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PROVISIONAL APPLICATION FOR PATENT COVER SHEET (Large Entity)

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

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☐ *Additional inventors are being named on page 2 attached hereto*

TITLE OF THE INVENTION (280 characters max)

STEREOSELECTIVE SYNTHESIS OF NUCLEOSIDE ANALOGUES

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ENCLOSED APPLICATION PARTS (*check all that apply*)

☒ Specification — *Number of Pages* 46

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☐ Other (specify)

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Respectfully submitted,

SIGNATURE [signature]

DATE FEBRUARY 12, 1999

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PROVISIONAL APPLICATION

OF

ROMAS KAZLOUSKAS

LANA JANES

ALEX CIMPOIA

FOR

UNITED STATES PATENT

ON

STEREOSELECTIVE SYNTHESIS OF NUCLEOSIDE ANALOGUES

CERTIFICATE OF MAILING BY "EXPRESS MAIL" (37 CFR 1.10)

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Docket No.: P 39 42393

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STEREOSELECTIVE SYNTHESIS OF NUCLEOSIDE ANALOGUES

FIELD OF THE INVENTION

The present invention relates generally to a novel method for the preparation of nucleoside analogues and their precursors and more particularly to a method of preparing a nucleoside analog by the use of specific enzymes to stereoselectively produce dioxolane nucleoside analogues or their precursors.

BACKGROUND OF THE INVENTION

The pharmacological activity of pharmaceutical compounds(drugs) depend mainly on their interaction with biological matrices (drug targets), such as proteins (receptors and enzymes), nucleic acids (DNA and RNA) and biomembranes(phospholipids and glycolipids). All these drug targets have complex three-dimensional structures which are capable of binding specifically to the drug in only one of the many possible arrangements in the three-dimensional space. It is the three-dimensional structure of the drug target that in part, determines which of the potential drug is bound within its cavity and with what affinity.

The spatial arrangement of atoms in an asymetric molecule is termed chirality. Chirality results in the creation of stereoisomers. Stereoisomers are compounds with identical chemical composition and atom connectivity (i.e. same constitution), but different arrangements of the atoms in space (i.e. different configurations). Stereoisomers are classified according to the number of chiral centers in each molecule and the spatial arrangement of the chiral center.

Chiral centers of organic molecules include chiral carbon atoms which have four different substituents connected

thereto and arranged in a generally tetrahedral configuration. Another type of chiral center is a chiral plane oriented along a rigid C=C bond that has at least two different substituents connected to the remaining four bond positions in that arrangement.

The chirality of molecules that are the subject of the present application refer to chirality created by chiral atoms and not chiral bonds. The following discussions will be limited to chirality created at one or more chiral carbon atoms which have four different substituents bound to each of the four different binding sites of the carbon.

When a molecule has a single chiral carbon, there are two stereoisomers that are mirror images of each other. This pair of isomers is termed enantiomers or an enantiomeric pair. When there are two chiral carbon atoms, there are four stereoisomers and two pairs of mirror images or enantiomers. A stereoisomer which is not a mirror image of another stereoisomer is a diastereisomer.

One type of stereochemical distinction relates to cyclic sugars or analogs of cyclic sugars. Cyclic sugars can be designated as a particular anomer depending upon the stereochemical configuration.

The term "anomer" designates the spatial arrangement of cyclic sugars or analogs or derivatives thereof that has two chiral centers in a five or six member ring. The ring is generally planar. The anomeric designation defines the relative configuration of the two chiral centers relative to a hypothetical plane defined by the ring. The chiral centers typically have two substituents outside the ring. One substituent is a H. The other substituent is a larger moiety such as a hydroxyl, methoxyl, purine or pyrimidine base, carboxyl, etc.

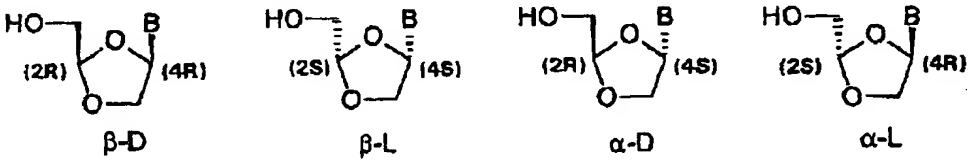
When the two larger constituents on each chiral center are on the same side of the plane in the ring, they are defined as a β-anomer (cis-isomer). When two larger moieties are on opposite sides of the planar ring, they are defined as the α-anomer (trans-isomer). An anomer is a type of diastereomer.

Because chirality is usually linked with biological activity or toxicity, it is important from the point of view of drug development to evaluate the physiological effect of each isomers. Frequently, one stereoisomer is considerably more active then the other. In other situations, the non-active isomer may inhibit the activity of the more active form. In some instances, the less preferred stereoisomer may be equally potent but have greater toxicity than the preferred stereoisomer. In each of these instances, the therapeutic effect of a drug can be increased if the single most preferred stereoisomer is administered in higher purity.

The current trend in the drug markets reflects a greater use of single stereoisomer drugs. The sales of single stereoisomer drugs have increased considerably. In 1995, single stereoisomer drugs sold for $61 billion worldwide. In the year 2000, that annual worldwide sales are expected to be $90 billion.

An important class of pharmacological agents relate to 3'-oxa-substituted 2',3'-dideoxynucleoside analogues ("dioxolane nucleoside analogues"). These compounds have two chiral centers corresponding to the substituted carbons 2 and 4 of the dioxolane ring (C2 and C4 respectively). Thus each compound can exist as four different stereoisomers depending on the position of both substituents with respect to the dioxolane ring.

The stereoisomers of a dioxolane nucleoside analog are represented by the following diagrams where the letter B represents a purine or pyrimidine base or an analogue or derivative of a purine or pyrimidine base as defined herewith.



For the purpose of consistency, the same stereochemical designation is used when the methoxyl moiety or the base moiety is replaced with another substituent group.

The C2 carbon in each of the above formulae is the carbon atom in the ring that connects the methoxy group to the dioxolane ring. The C4 carbon is the carbon atom in each of the above formulae that connects the base (B) substituent to the dioxolane ring.

The four stereoisomers represented above are two diastereomer i.e. pairs of two enantiomers. The β-anomers represent one set of enantiomers, the β-L enantiomer and the β-D enantiomer. The α-anomers represent one set of enantiomers, the α-L enantiomer and the α-D enantiomer.

Compounds with D-configuration have an outward directed methoxy group when the ring is oriented in the plane of the page with the oxygen in the first position (O3) at the top of the page with the carbon in the two position (C2) on the left side as illustrated above. This is also represented by the designation (2R). Compounds having an L-configuration has inward directed methoxy group when the ring is oriented in the plane of the page with the O3

oxygen at the top of the page with the C2 carbon on the left side as illustrated above. This is also represented by the designation (2S).

A variety of dioxolane nucleoside analogues have been identified to have antiviral and anticancer activity. For example, 9-(β-D-2-hydroxymethyl-1,3-dioxolan-4-yl)-2-aminopurine (β-D-DAPD) and its metabolite 9-(β-D-2-hydroxymethyl-1,3-dioxolan-4-yl)-guanine (β-D-DXG) have been reported to have potent and selective activity against human immunodeficiency virus (HIV) and hepatitis B virus (HBV) (Rajagopalan et al., Antiviral Chem. Chemother.,1996, 7(2), 65-70) Similarly, 1-(β-L-2-hydroxymethyl-1,3-dioxolan-4-yl)-thymine (Dioxolane-T)(Norbeck et al., Tetrahedron Lett., 1989,30, 6263-66) possess anti-HIV and anti-HBV activity. 1-(β-D-2-hydroxymethyl-1,3-dioxolan-4-yl)-cytidine (β-L-OddC) (Bednarski et al., Bioorg. Med. Chem. Lett., 1994, 4, 2667-72) was discovered to have potent anti-tumor activity towards human prostate as well as renal carcinoma (Kadhim et al., Can. Cancer Res., 57(21),4803-10, 1997). β-L-OddC is the first nucleoside analogue with an L-configuration shown to have anticancer activity. Since stereoisomers of dioxolane nucleosides usually have different biological activities and toxicity, obtaining the pure therapeutically active isomer becomes crucial.

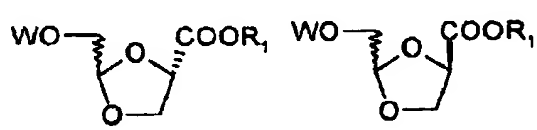
Chiral synthetic methods have improved over the past several years with respect to synthetic techniques that result in single stereoisomer compounds. However, there is a present need to find novel synthetic methods which can be widely used to form a particular stereoisomer with greater efficiency and purity.
For examples, for many years a person of ordinary skill in the art could use enzymes to separate enantiomers of

dioxolane compounds. However, it is not known in the art how to produce a dioxolane nucleoside analog using a step of separating an anomeric mixture of certain dioxolane precursors to produce an end product with greater efficiency and purity.

Because stereochemically pure dioxolane nucleosides are an important class of compounds due to their known antiviral activity against retroviruses, such as human immunodeficiency viruses (HIV) and hepatitis B virus (HBV) and activity against various types of cancers, there is a need for other inexpensive and efficient stereoselective methods for their preparation. The present invention satisfies this and other needs.

SUMMARY OF THE INVENTION

The present invention is a novel process for making a class of dioxolane nucleoside analogs that include a step of separating with certain enzymes β and α anomers from an anomeric mixture of dioxolane nucleoside analogue precursors which provides higher yield and greater efficiency of producing dioxolane nucleoside analogs. Specifically, the present invention is a process for making dioxolane nucleoside analogs and includes the step of separating β and α anomers from an anomeric mixture represented by the following formula A or formula B:



(A) (B)

wherein W is benzyl or benzoyl and R_1 is selected from the group consisting of C_{1-6} alkyl and C_{6-15} aryl. The process involves the step of hydrolyzing the mixture of compounds represented by formula A and formula B with an enzyme

selected from the group consisting of cholesterol esterase, ESL-001-02, horse liver esterase, bovine pancreatic protease, α-chymotrypsin, protease from streptomyc s caeopitosis, substilisin from bacillus licheniformis, protease from aspergillus oryzae, proteinase from bacillus licheniformis, subtilisin from bacillus licheniformis, protease from streptomyces griseus, acylase from aspergillus melletus, proteinase from bacillus subtilis, diversa clonezyme #5, pronase from streptomyces, lipase from rhizopus arrhizus, lipoprotien lipase from pseudomonas sp. type B, bacterial proteinase, lipase from pseudomonas cepacia and bacterial protienase. The process stereoselectively hydrolyses predominantly one anomer to form a product where R_1 of formula A and formula B is replaced with H. The other anomer remains substantially unhydrolyzed. The process also comprises separating the hydrolyzed product from unhydrolyzed starting material.

The process of one embodiment further includes the step of stereoselectively replacing the R_1 group with a purinyl, pyrimidinyl or derivative thereof to produce a dioxolane nucleoside analog that has a high degree of stereochemical purity.

According to one embodiment of the invention, the step of hydrolyzing results in a starting material having an anomeric purity of at least 80%. According to another embodiment, the step of hydrolyzing results in a starting material having an anomeric purity of at least 90%. In yet another embodiment, the step of hydrolyzing results in a starting material having an anomeric purity of at least 95%. In an additional embodiment, the step of hydrolyzing results in a starting material having an anomeric purity of at least 98%.

According to one embodiment of the invention, the step of hydrolyzing results in a product having an anomeric

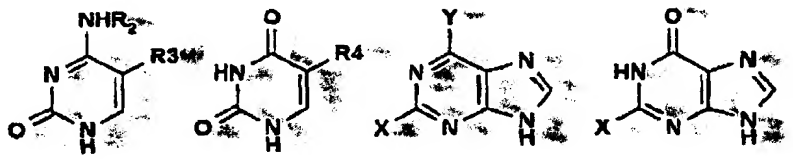
purity of at least 80%. According to another embodiment, the step of hydrolyzing results in a product having an anomeric purity of at least 90%. In yet another embodiment, the step of hydrolyzing results in a product having an anomeric purity of at least 95%. In an additional embodiment, the step of hydrolyzing results in a product having an anomeric purity of at least 98%.

In one embodiment of the present invention, W of formula A or formula B is benzyl and the enzyme is selected from the group consisting of cholesterol esterase, ESL-001-02, horse liver esterase, bovine pancreatic protease, α-chymotrypsin, protease from streptomyces caeopitosis, subtilisin from bacillus licheniformis. In another embodiment, the enzyme is α-chymotrypsin. In yet another embodiment, the enzyme is bovine pancreatic protease.

In one embodiment of the present invention, W of formula A and formula B is benzoyl and the enzyme is selected from the group consisting of protease from aspergillus oryzae, proteinase from bacillus licheniformis, subtilisin from Bacillus licheniformis, protease from streptomyces griseus, acylase from aspergillus melletus, proteinase from bacillus subtilis, diversa clonezymes #5, pronase from streptomyces, lipase from Rhizopus arrhizus, lipoprotein lipase from pseudomonas sp. type B, bacterial protienase, and lipase from pseudomonas cepacia. In another embodiment, the enzyme is selected from the group consisting of aspergillus oryzae, proteinase from bacillus licheniformis, subtilisin from Bacillus licheniformis, protease from streptomyces griseus, pronase from streptomyces, and lipase from rhizopus arrhizus. In yet another embodiment, the enzyme is selected from the group comprising aspergillus oryzae and proteinase from bacillus licheniformis.

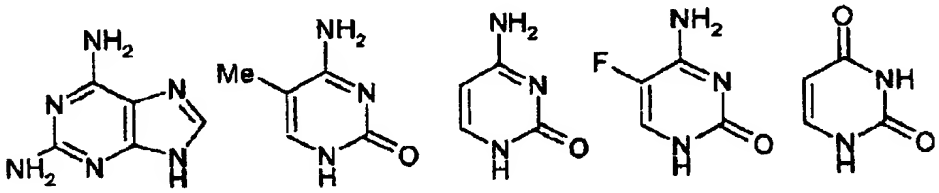
In one embodiment, the β-anomer is the predominant product. In another embodiment, the α-anomer is the predominant product. In yet another embodiment, the β-L-enantiomer is the predominant product. In an additional embodiment, the β-D-enantiomer is the predominant product. In yet another embodiment, the α-L-enantiomer is the predominant product. In an additional embodiment, the α-D-enantiomer is the predominant product.

In one embodiment, the invention is a process for stereoselectively preparing a dioxolane nucleoside analog by separating β and α-anomers from an anomeric mixture represented by formula A or formula B according to one of the above embodiments. The process further includes the step of stereoselectively replacing the R_1 group with a purinyl or pyrimidinyl or derivative selected from the group consisting of:



In this embodiment, R_2 is selected from the group consisting of hydrogen, C_{1-6} alkyl, C_{1-6} acyl and $R_5C(O)$ wherein R_5 is hydrogen or C_{1-6} alkyl. Additionally, R_3 and R_4 are each independently selected from the group consisting of hydrogen, C_{1-6} alkyl, bromine, chlorine, fluorine, and iodine; and X and Y are each independently selected from the group consisting of hydrogen, bromine, chlorine, fluorine, iodine, amino, and hydroxyl. The process results in the production of a stereochemical isomer of the dioxolane nucleoside analog or derivative thereof.

In another embodiment, the process comprises stereoselectively preparing a dioxolane nucleoside analog by separating β and α anomers from an anomeric mixture represented by formula A or formula B according to one of the above embodiments and further comprises stereoselectively replacing the R group with a moiety selected from the group consisting of:



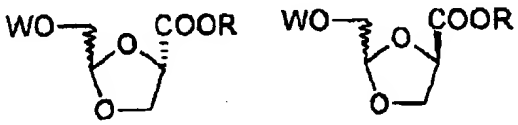
In another embodiment of the present invention, the process comprises making a dioxolane nucleoside analog by separating a compound according to formula A or formula B. According to this embodiment, the process includes stereoselectively replacing the R group with a purinyl or pyrimidinyl moiety or derivative thereof by acylating the second mixture to produce an acylated second mixture. This embodiment also includes the step of glycosylating the acetylated second mixture with a purine or pyrimidine base or derivative thereof and a Lewis Acid to produce the dioxolane nucleoside analog.

DETAILED DESCRIPTION OF THE INVENTION

The present invention involves a high yield process of separating β and α anomers from an anomeric mixture of dioxolane nucleoside analogue precursors which provides higher yield and greater efficiency. In one embodiment, this method is used in the production of dioxolane nucleoside analogs having a high degree of steric purity at lower cost. Additionally, another aspect of the present invention involves synthesizing starting material having a higher degree of anomeric purity.

The present invention provides a process of preparing dioxolane nucleoside analogues having a predominant β-L-configuration using stereoselective enzymes, namely hydrolases. The procedure improves overall yield and has relatively few steps which improves overall efficiency. The process involves the following steps.

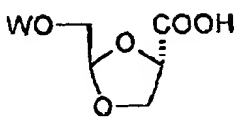
A mixture of anomers represented by formula A or formula B is obtained.



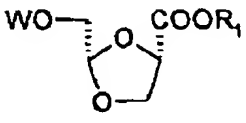
(A) (B)

In the above formula, W is benzyl or benzoyl, and R_1 is selected from the group consisting of H, C_{1-6} alkyl and C_{6-15} aryl. The mixture is hydrolyzed with an enzyme selected from the group consisting of cholesterol esterase, ESL-001-02, horse liver esterase, bovine pancreatic protease, α-chymotrypsin, protease from streptomyces caeopitosis, substilisin from bacillus licheniformis, protease from aspergillus oryzae, proteinase from bacillus licheniformis, subtilisin from bacillus licheniformis, protease from streptomyces griseus, acylase from aspergillus melletus, proteinase from bacillus subtilis, diversa clonezyme #5, pronase from streptomyces, lipase from rhizopus arrhizus, lipoprotein lipase from pseudomonas sp. type B, bacterial proteinase, lipase from pseudomonas cepacia and bacterial proteinase. The hydrolyzing step stereoselectively hydrolyzes the α-anomer of the mixture of either formula A or formula B. The result is an unhydrolyzed β-anomer. The α-anomer can be separated easily from the β-anomer. If an anomeric mixture of the compound of formula A is

selected, the result is the production of the compound of formula C and formula D:

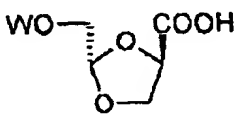


(C)

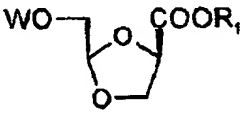


(D)

If an anomeric mixture of the compound of formula B is selected, the result is the production of the compound of formula E and formula F:



(E)



(F)

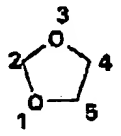
The mixture is then acylated to replace the R_1 group with an acyl moiety. Then, the mixture is glycosylated with a purine or pyrimidine base or derivative thereof and a Lewis Acid. The final step produces a dioxolane nucleoside analogue in the β-L configuration.

At the outset, the following definitions have been provided as reference. Except as specifically stated otherwise, the definitions below shall determine the meaning throughout the specification.

"Nucleoside" is defined as any compound which consists of a purine or pyrimidine base, linked to a pentose sugar.

"Dioxolane nucleoside analogue" is defined as any compounds containing a dioxolane ring as defined hereinafter linked to a purine or pyrimidine base or derivative thereof. A "dioxolane ring" is any substituted or unsubstituted five member monocyclic ring

that has an oxygen in the 1 and 3 positions of the ring as illustrated below:



Dioxolane Ring

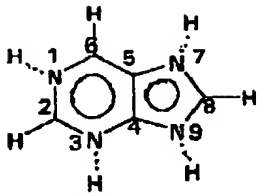
"Purine or pyrimidine base" is defined as the naturally occurring purine or pyrimidine bases adenine, guanine, cytosine, thymine and uracil. A purine or pyrimidine that is a moiety is a purinyl or pyrimidinyl, respectively.

"Alkyl, is defined as a substituted or unsubstituted, saturated or unsaturated, straight chain, branched chain or carbocyclic moiety, wherein the straight chain, branched chain or carbocyclic moiety can be optionally interrupted by one or more heteroatoms (such as oxygen, nitrogen or sulfur). A substituted alkyl is substituted with a halogen(F, Cl, Br, I), hydroxyl, amino or C_{6-20} aryl.

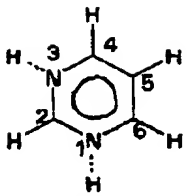
"Aryl, is defined as a carbocyclic moiety which can be optionally substituted or interrupted by one heteroatom (such as oxygen, nitrogen or sulfur) and containing at least one benzenoid-type ring (such as phenyl and naphthyl).

"Carbocyclic moiety" is defined as a substituted or unsubstituted, saturated or unsaturated, C_{3-6} cycloalkyl wherein a substituted cycloalkyl is substituted with a C_{1-6} alkyl, halogen (i.e. F. Cl, Br, I), amino or NO_2.

A "derivative" of a purine or pyrimidine base refers to one of the following structures:



Purine



Pyrimidine

wherein one ore more of the pyrimidine H are substituted with substituents that are known in the art. Substitutents bound to the ring members by a single bond include but are not limited to halogen such as F, Cl, Br, I; an akyl such as lower akyls; aryl; cyano carbamoyl; amino including primary, secondary and tertiary amino; and hydroxyl groups. Substituents bound to the carbon ring atoms by a double bond include but are not limited to a =O to form a carbonyl moiety in the ring. It is understood that when the ring is aromatic, some of the substitutions may form tautomers. The definition shall include such tautomers.

"Stereoselective enzymes" are defined as enzymes which participate as catalysts in a reaction that selectively yields one specific stereoisomer over other stereoisomers.

"Steric purity" is defined as the amount of a particular stereoisomer of a compound divided by the total amount of all stereoisomers of that compound present in the mixture multiplied by 100%.

"Anomeric purity" is defined as the amount of a particular anomer of a compound divided by the total amount of all anomers of that compound present in the mixture multiplied by 100%.

"Diastereomeric purity" is defined as amount of a particular diastereomer of a compound divided by the total amount of all diastereomers of that compound

present in the mixture multiplied by 100%.

"Alkoxy" is defined as an alkyl group, wherein the alkyl group is covalently bonded to an adjacent element through an oxygen atom (such as methoxy and ethoxy).

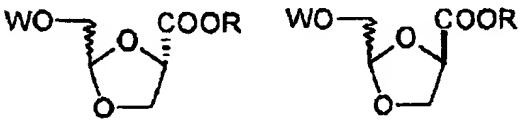
"Alkoxycarbonyl", is defined as an alkoxy group attached to the adjacent group of a carbonyl.

"Acyl" is defined as a radical derived from a carboxylic acid, substituted (by a halogen, C_{6-20} aryl or C_{1-6} alkyl) or unsubstituted by replacement of the -OH group. Like the acid to which it is related, an acyl radical may be aliphatic or aromatic, substituted (by halogen, C_{1-6} alkoxyalkyl, nitro or O_2) or unsubstituted, and whatever the structure of the rest of the molecule may be, the properties of the functional group remain essentially the same (such as acetyl, propionyl, isobutanoyl, pivaloyl, hexanoyl, trifluoroacetyl, chloroacetyl and cyclohexanoyl).

"Alkoxyalkyl" is defined as an alkoxy group attached to the adjacent group by an alkyl group (such as methoxymethyl).

"Acyloxy" is defined as an acyl group attached to the adjacent group by an oxygen atom.

As noted above, one embodiment of the present invention is a process for separating β and α anomers from an anomeric mixture represented by the following formula A or formula B:



(A)

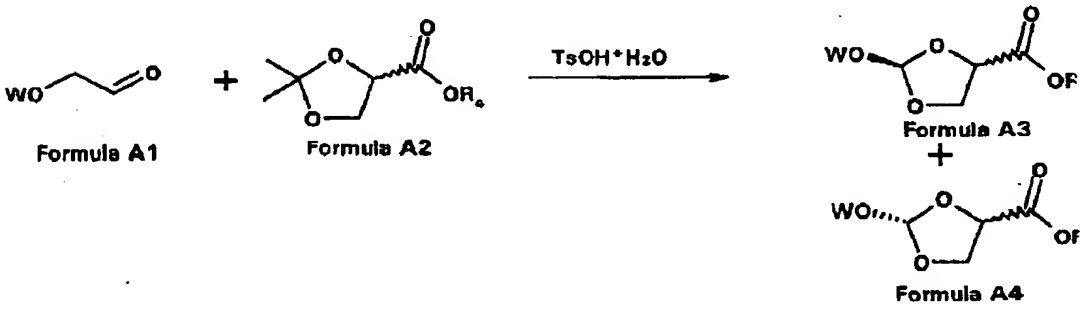
(B)

wherein W is benzyl or benzoyl and R_1 is selected from the group consisting of C_{1-6} alkyl and C_{6-15} aryl.

In one embodiment, the process stereoselectively hydrolyses predominantly the α-anomer to form a product where R_1 of formula A and formula B is replaced with H. The β-anomer remains substantially unhydrolyzed. The process also comprises separating the hydrolyzed product from unhydrolyzed starting material.

The process of making a β-L dioxolane nucleoside analog begins with the preparation of starting materials. Scheme 1 depicts the manufacture of a mixture that includes formula A or B.

Scheme 1



An oxyacetaldehyde represented by formula 1A (wherein W is benzyl or benzoyl) is reacted with 1,3-Dioxolane-4-Carboxylic acid-2,2-dimethyl-methyl ester (formula 1B) in approximately equimolar proportions. The dioxolane of formula 1B has a chiral center at the C4 carbon. The reaction occurs in a toluene solvent. The mixture his heated to 58°C. The catalyst, $TsOH \cdot H_2O$, is added. The mixture is heated to a temperature between 64-67°C. A vacuum is applied at 70 kPa, and the reaction proceeds for 40 minutes. Traces of solvent are then removed by

high vacuum. The catalyst his removed by filtration using a 1:1 ratio of Hexane:EtOAc as an eluent. In one embodiment, the preferred filter is a silica gel pad. The resulting product is a crude oil containing a mixture of the compounds of formula 1C and 1D wherein the ratio is 2:1 of (1C:1D), respectively.

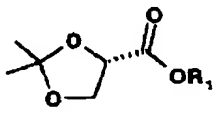
It can be appreciated by a person of skill in the art that the reaction conditions can be adjusted to optimize the purity of the stereoisomers. In one embodiment of the present invention, the reaction of the compound of formula 1A with the compound of formula 1B is done in the presence of catalyst in an amount between about 2.5 wt% and about 5.5 wt% of the starting materials. In yet another embodiment, the amount of catalyst is between about 3.0 wt% and about 5.0 wt%. In still another embodiment, the amount of catalyst is between about 3.5%. and about 5.5%.

In an embodiment of the present invention, the reaction of the compound of formula 1A with the compound of formula 1B is done at a temperature ranging from about 40^0C to about 80^0C. In another embodiment of the present invention, the temperature ranges from about 50^0C to about 75^0C. In still another embodiment, the temperature ranges from about 60^0C to about 70^0C. In an additional embodiment, the temperature ranges from about 65^0C to about 79^0C.

In an embodiment of the present invention, the reaction time between the compound of formula 1A and the compound of formula 1B corresponds to a period ranging from about 30 minutes to about 2 hours. In yet another embodiment, the period ranges from about 30 minutes to about 1 hour. In still another embodiment, the period ranges from about 30 minutes to about 50 minutes.

It will be appreciated by a person of ordinary skill in the art that the C4 carbon is chiral. Because this carbon is not involved in the reaction, the chirality is preserved at that carbon. A starting material can be selected to have a (4S) or (4R) stereochemistry. It is important to know that the above reaction favors one configuration.

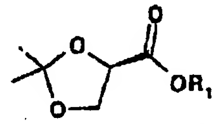
According to one embodiment, it is preferable that the resulting product represented by formula 1C and 1D represent an anomeric mixture having a high anomeric purity of a mixture of only two anomers. To achieve such a result, the starting material represented by formula 1B is selected to have a single stereoisomer at the 4 carbon. By way of example and not by limitation, the starting material represented in Scheme 1 by formula 1B has 4S configuration as shown below in the following formula 1B(4S):



Formula 1B (4S)

The reaction proceeds according to the principles described above. The resulting product will have an anomeric purity of the β-L anomer over the α-L anomer of greater than 55%, preferably 60% and more preferably 65%.

According to another embodiment, it is preferable to have a product with higher anomeric purity of the α-D-anomer over the β-D-anomer. The starting material represented in Scheme 1 by formula 1B has 4S configuration as shown below in the following formula 1B(4S).

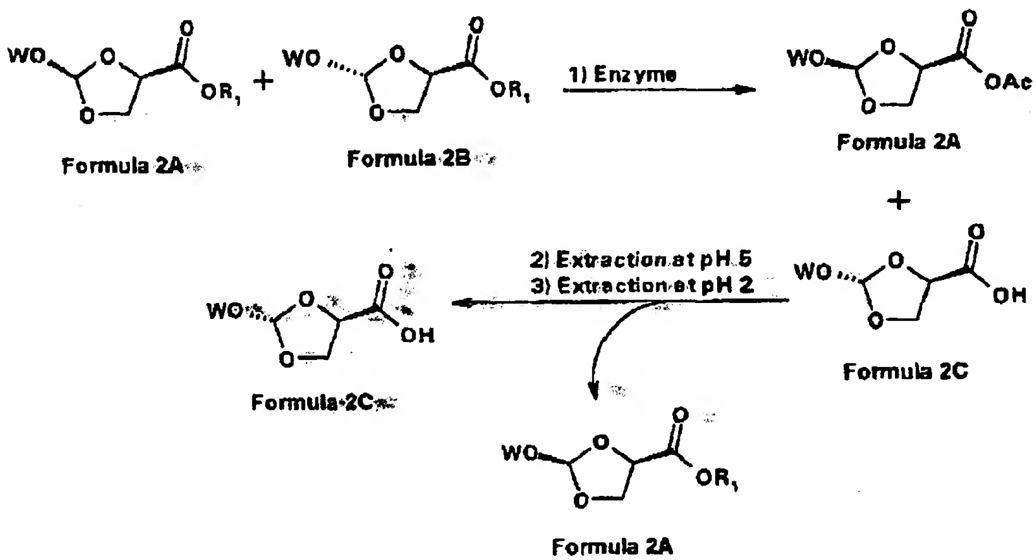


F rmula 1B (4R)

The reaction proceeds according to the principles described above. The resulting product will have an anomeric purity of the α-D-anomer greater than 55%, preferably, 60% and more preferably 65%.

According to one embodiment, the present invention is a method of separating β-anomers from α-anomers according to the following Scheme 2:

Scheme 2



According to one embodiment, a mixture of anomers is obtained as represented by formula 2A or formula 2B. A mixture represented by formula 2A or formula 2B can be obtained according to the reaction described above or according to any method known in the art.

The reaction is prepared as follows: A portion of the material represented by formula 2A and formula 2B is weighed into a reaction vessel. For every 0.001 mmol of the mixture of formula 2A and formula 2B added to the reaction vessel, between 10 ml and 13 ml of a 5 mmol solution of BES buffer is added. Preferably, between 11 ml and 19 ml, and most preferably 11.5 ml, of a 5 mmol solution of a BES buffer is added for each mmol of compound. The pH of the buffer should be between 7.0 and 7.5 and preferably 7.2.

The enzyme is selected from the group consisting of cholesterol esterase, ESL-001-02, horse liver esterase, bovine pancreatic protease, α-chymotrypsin, protease from streptomyces caecpitosis, substilisin from bacillus licheniformis, protease from aspergillus oryzae, proteinase from bacillus licheniformis, subtilisin from bacillus licheniformis, protease from streptomyces griseus, acylase from aspergillus melletus, proteinase from bacillus subtilis, diversa clonezyme #5, pronase from streptomyces, lipase from rhizopus arrhizus, lipoprotein lipase from pseudomonas sp. type B, bacterial proteinase, lipase from pseudomonas cepacia and bacterial proteinase.

The commercial sources of the enzymes are readily available to a person of ordinary skill in the art. Particularly, some of the materials can be obtained from the following sources: Bovine bacterial proteinase was purchased from Genzyme (Cambridge, MA); ESL-001-02 from Diversa Corp. (San Diego, CA); Horse liver esterase, subtilisin and Bacillus licheniformis from Fluka Chemie (Oakville, ON); Bovine pancreas type 1, α-chymotrypsin and streptomyces caeopitosis from Sigma-Aldrich (Oakville, ON).

In another embodiment, the enzyme is selected from the group consisting of protease from aspergillus oryzae,

proteinase from bacillus licheniformis, subtilisin from Bacillus licheniformis, protease from streptomyces griseus, acylase from aspergillus melletus, proteinase from bacillus subtilis, diversa clonezymes #5, pronase from streptomyces, lipase from Rhizopus arrhizus, lipoprotein lipase from pseudomonas sp. type B, bacterial proteinase, lipase from pseudomonas cepacia. The selection of one of these enzymes is preferred according to this embodiment when the oxyacetaldehyde represented by the compound of formula 1A in Scheme 1 is selected to be benzoyloxyacetaldehyde.

According to another embodiment of the invention, the oxyacetaldehyde represented by the compound of formula 1A is benzoyloxyacetaldehyde. According to this embodiment, the enzyme is selected from the group consisting of aspergillus oryzae, proteinase from bacillus licheniformis, subtilisin from Bacillus licheniformis, protease from streptomyces griseus, pronase from streptomyces, and lipase from rhizopus arrhizus. In yet another embodiment, the enzyme is selected from the group consisting of aspergillus oryzae and proteinase from bacillus licheniformis.

In yet another embodiment, the enzyme is selected from the group consisting of cholesterol esterase, ESL-001-02, horse liver esterase, bovine pancreatic protease, α-chymotrypsin, protease from streptomyces caeopitosis and substilisin from bacillus licheniformis. The selection of one of these enzymes is preferred according to this embodiment when the oxyacetaldehyde represented by the compound of formula 1A is benzyloxyacetaldehyde.

The stereospecific enzyme selected is then added to begin the hydrolysis reaction. The enzymatic reaction hydrolyzes primarily the α-anomer by replacing the R_1 group of the α-anomer of the compound of formula 2B with

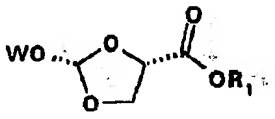
H to form the compound of formula 2C. The amount of the enzyme added can be determined according to principals known by any person of ordinary skill in the art. According to another embodiment, about 50 mL was added to begin the reaction. The rate and degree of hydrolysis was monitored by a pH-stat according to principles known in the art. As the compound of formula 2B is hydrolyzed, the pH of the mixture decreases. Thus, the pH corresponds to the completeness of the reaction.

If the reaction time is allowed to proceed longer than the optimal reaction time, the β-anomer may be converted resulting in lower anomeric purity of the final product. If the reaction time is too short, less than optimal amount of the α-anomer is converted resulting in a lower anomeric purity of the remaining unhydrolyzed reactant. According to one embodiment, the reaction is allowed to proceed until 43% completion. This occurs when the pH is 5. According to this embodiment, there is a higher stereospecific purity of the converted products (i.e. the compound of formula 2C). However, it will be appreciated by a person of ordinary skill in the art that the exact degree of completion may change depending upon the reactant used, the enzyme used and other principles known to a person of ordinary skill in the art.

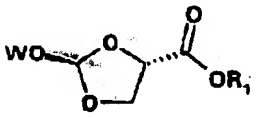
As noted, the ester starting material is separated from the hydrolyzed acid product when the desired endpoint is reached. According to one embodiment, the endpoint occurs at a pH of approximately 5. The ester starting material is extracted from the aqueous solution with ethyl acetate (for example, 3 x 20 mL). The pH of the solution is then adjusted to pH 2. The hydrolyzed product is extracted with ethyl acetate (for example, 3 x 20 mL). The reactants and the products are dried with $MgSO_4$, filtered and concentrated *in-vacuo*. Because of the enzyme selectivity, the anomeric purity of the hydrolyzed

and separated α-anomer is considerable. Furthermore, the anomeric purity of the unhydrolyzed and separated α-anomer is also considerable. In one embodiment, the anomeric purity of the respective separated α-anomer and/or the β-anomer is greater than 80%. In another embodiment, the anomeric purity of the respective separated α-anomer and/or the β-anomer is greater than 90%. In another embodiment, the anomeric purity of the respective separated α-anomer and/or the β-anomer is greater than 95%. In another embodiment, the anomeric purity of the respective separated α-anomer and/or the β-anomer is greater than 98%.

In another embodiment, the procedure of Scheme 2 is followed except the anomeric mixture represented by formula 2A and 2B is replaced with an anomeric mixture represented by formula 2D and 2E, respectively.



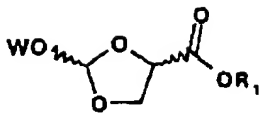
Formula 2D



Formula 2E

According to this embodiment the α-anomer represented by formula 2E is hydrolyzed. The result is the separation of the hydrolyzed α-anomer represented by formula 2E from the unhydrolyzed β-anomer represented by formula 2D.

In another embodiment, the procedure of Scheme 2 is followed except a mixture represented by formula 2A and 2B is replaced with a mixture of four stereoisomers represented by formula 2F.



Formula 2F

According to this embodiment, the α-anomer containing both D and L enantiomers is hydrolyzed. The result is the separation of the hydrolyzed α-anomer containing both D and L enantiomers from the unhydrolyzed β-anomer containing both D and L enantiomers.

After hydrolysis and purification, the selected purified anomer can be linked with a purine or pyrimidine base or derivative. There are several examples known by skilled artisan on how to link a purine or pyrimidine base or derivative to the dioxolane ring. For example, PCT Publ. No. WO/97/21706 by Mansour et al. describes one method of stereoselectively attaching the purine or pyrimidine base to a dioxolane ring: WO/97/21706 is incorporated herein fully by reference.

According to the process disclosed in WO/97/21706 the starting material is an acylated dioxolane ring. Acylation can be performed according to the principles of the present invention. Acylation, destroys the stereochemistry of the C2 carbon while preserving the stereochemistry of the C4 carbon. Therefore, if it is desirable to have a final end product with specific stereochemistry on the C2 carbon, the procedure subsequent to the acylation step must stereoselectively produce a product having a particular stereochemistry at the C2 carbon. The procedure disclosed in WO/97/21706 accomplishes this objective.

As noted, the acylation step occurs after the hydrolysis step. A compound having the desired stereochemistry on the C2 carbon is selected. For each mmol of compound

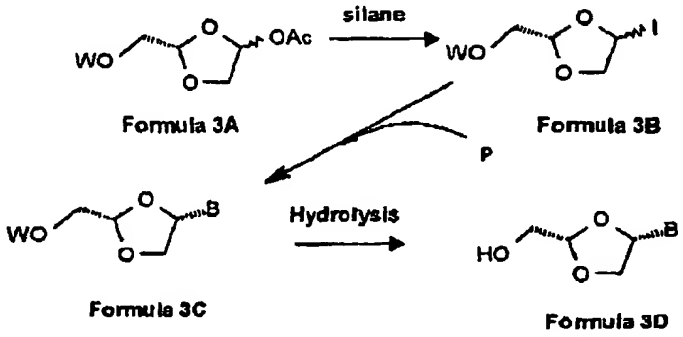
that is processed, it is dissolved in between about 2.5 and about 4.0 mL of acetonitrile. In another embodiment, between about 3.0 and about 3.5 mL of acetonitrile was added for each mmol of compound. In yet another embodiment, between about 3.3 and about 3.4 mL of acetonitrile was added for each mmol of compound.

For each mmol of compound, between about 0.08 and about 0.12 ml of pyridine was added. In another embodiment, between about 0.09 and about 0.11 mL of pyridine was added for each mole of compound. In yet another embodiment, approximately 0.1 ml of pyridine was added for each mmol of compound.

To this mixture, between 1.1 and 1.5 mmoles of $Pb(OAc)_4$ was added for each mmol of compound. In another embodiment, between about 1.2 mmoles and about 1.4 mmoles of $Pb(OAc)_4$ is added for each mmol of compound. In yet another embodiment, about 1.3 mmoles of $Pb(OAc)_4$ is added for each mmol of compound.

Thereafter, the mixture was stirred for 18 hours at room temperature. Then, the mixture was poured into a saturated solution of $NaHCO_3$. Between approximately 2.0 and 3.0 mL of $NaHCO_3$ were used for each mmol of compound. In one embodiment, between about 2.5 mL and about 2.7 mL, and more preferably about 2.6 ml of $NaHCO_3$ was used for each mmol of compound. The solution was then stirred for an additional 30 minutes. The organic layer was separated from the aqueous layer by four extractions of ethyl acetate. Extracts were combined, dried on anhydrous Na_2SO_4 and evaporated under a vacuum. Optionally, the crude can be further purified by chromatography on silica gel using a gradient of 0-15% Ethyl acetate in hexane.

In one embodiment of the present invention, the acylation step is followed by glycosylation. The glycosylation is represented by the following Scheme 3.

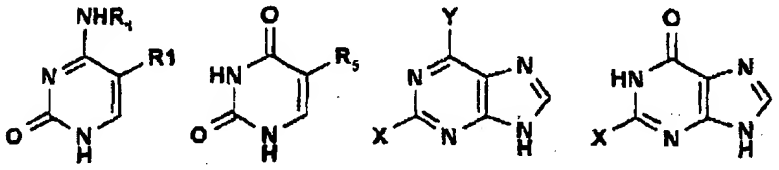


The first step in the glycosylation procedure is to obtain a compound with the desired stereospecificity at the C2 carbon. According to one embodiment, a compound having an S stereochemistry at the C2 carbon, as represented by the compound of formula 3A is preferred. The result is that a higher ratio of the β-anomer is in the product 3C. According to another embodiment, compound having an R stereochemistry at the C2 carbon is preferred. The result is a product that has a higher ratio of the α-anomer in the final product.

The compound of formula 3A is reacted with a silane compound to produce the compound of formula 3B. In one embodiment, the silane compound is iodotrimethylsilane. In another embodiment, the silane compound is diiodosilane. Important to the reaction is that it occurs at low temperatures. According to one embodiment, the temperature is preferably 78°C prior to glycosylation with silylated pyrimidine or purine base or derivative thereof.

The iodo intermediate represented by formula 3B is then dissolved in dichloromethane and is cooled down to -78° C. A purine or pyrimidine base is then selected. According

to one embodiment, the purine or pyrimidine base or derivative thereof is selected from the following group:



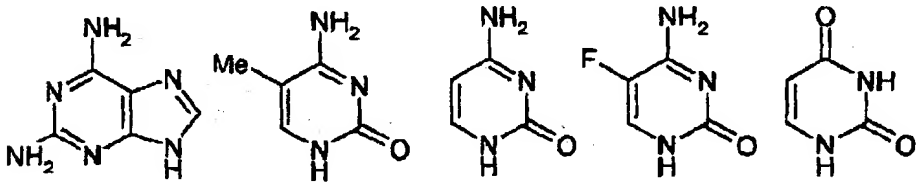
wherein

R_2 is selected from the group consisting of hydrogen, C_{1-6} alkyl, C_{1-6} acyl and $R_5C(O)$ wherein R_5 is hydrogen or C_{1-6} alkyl;

R_3 and R_4 are each independently selected from the group consisting of hydrogen, C_{1-6} alkyl, bromine, chlorine, fluorine, and iodine; and

X and Y are each independently selected from the group consisting of hydrogen, bromine, chlorine, fluorine, iodine, amino, and hydroxyl.

In another embodiment of the invention, the purine or pyrimidine base or derivative thereof is selected from the group consisting of:



The purine or pyrimidine or derivative thereof is persylated by a silating agent and ammonium sulphate followed by evaporation of HMDS to form a persylated purine or pyrimidine base or derivative thereof herein referred to as the persylated base and designated as P in Scheme 3. According to one embodiment, the sylating agent is selected from the group consisting of 1,1,1,3,3,3-hexamethyldisilazane, trimethylsilyl

triflate, t-butyldimethylsilyl triflate or trimethylsilyl chloride. In one embodiment, the sylating agent is 1,1,1,3,3,3,-hexamethyldisilazane.

The persylated base P was dissolved in 30 mL of dichloromethane and was added to the iodo intermediate represented by formula 3B. The reaction mixture was maintained at -78°C for 1.5 hours then poured onto aqueous sodium bicarbonate and extracted with dichloromethane (2x25 mL). The organic phase was dried over sodium sulphate to obtain the compound of formula 3C. As used in Scheme 3, the B represents a moiety of the purine or pyrimidine base or derivative thereof which was persylated in the above step to form P. The compound of formula 3C was removed by filtration and the solvent was evaporated *in-vacuo* to produce a crude mixture. The product represented by formula 3C has predominantly a S stereochemistry on the C4 carbon with an anomeric purity of 80%. When the starting material is a compound represented by formula 3A, the product forms predominantly the β-L enantiomer having an anomeric purity of 80%.

Next, the compound of formula 3C is hydrolyzed to produce the compound of formula 3D. This is accomplished by dissolving a compound represented by formula 3C in EtOH and then adding cyclohexene and palladium oxide. The reaction mixture is refluxed for 7 hours. It is then cooled and filtered to remove solids. The solvents are removed from the filtrate by vacuum distillation. The product represented by formula 3D is purified by flash chromatography on silica-gel (5% MEOH in EtOAc).

Example 1. Enzyme catalyzed hydrolytic resolution of the dioxolane methyl ester.

A 2:1 (β:α) anomeric mixture of (2-(S)-benzyloxymethyl)-4-carboxylic acid-1,3-dioxolane methyl ester) (136.5 mg,

0.541mmol) was weighed into a reaction vessel and BES buffer(6,263 mL of a 5 mM solution, pH 7.2) was added. The substrate remained as insoluble droplets. α-Chymotrypsin (500 mmol, 0.019 units by PNPA assay) was added to begin the reaction and the rate and degree of hydrolysis was monitored by a pH-stat which maintained the pH at 5 by automatic titration with 0.0981 mmol NA⊙H. The reaction was terminated at 43% conversion for high anomeric purity as determined by Sih's equations for recycling, by extracting the remaining starting material ester with ethyl acetate (3x20mL). The aqueous layer was adjusted to pH 2 and the product acid extracted with ethyl acetate (3x20mL).

Both extracts were dried with $MgSO_4$, filtered and concentrated *in-vacuo*. By this method, we obtained the (2-(S)-benzyloxymethyl)-4-(S)-carboxylic acid-1,3-diaxolanemethyl ester).

Example 2. Purity of β-Anomer by NMR.

Analysis was performed on a Varian Gemini 200 MHz NMR spectrometer in $CDCl_3$. The α-ester shows a triplet at d 5.33 (3j = 4.6 Hz) and the β-ester shows a triplet upfield at d 5. 23 (3 J = 4. 6 Hz). The β-acid shows a triplet at d 5.33(3J = 3.6 Hz), while the β-acid shows a broad singlet upfield at d 5.19. We did not observe any epimerization of the substrate or product acid during work-up. The purity of the β-anomer is determined by Sih's equation to have 98% anomeric purity.

Example 3: Purity of the α-Anomer.

The product acid is obtained from example 2 after it was dried with $MgSO_4$, filtered and concentrated *in-vacuo*. It is analyzed for purity by NMR. The purity is calculated

by Sih's equation. The α-anomer is isolated with high steric purity.

Example 4: Enzymatic Resolution of β-anomer with Protease from Aspergillus oryzae.

The procedure of Examples 1-2 were followed using protease from Aspergillus oryzae as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-carboxylicacid-1,3-dioxolane methyl ester). The result is a β-anomer that has high diastereomeric purity.

Example 5: Enzymatic Resolution of α-anomer with Protease from Aspergillus oryzae.

The product acid is obtained from Example 4 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high steric purity.

Example 6: Enzymatic Resolution of β-anomer with Proteinase from Bacillus Licheniformis.

The procedure of Examples 1-2 were followed using proteinase from bacillus licheniformis as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-carboxylicacid-1,3-dioxalane methyl ester). The result is a β-anomer that has high diastereomeric purity.

Example 7: Enzymatic Resolution of α-anomer with Proteinase from Bacillus Licheniformis.

The product acid is obtained from Example 6 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high steric purity.

Example 8: Enzymatic Resolution of β-anomer with Subtilisin from Bacillus Licheniformis.

The procedure of Examples 1-2 were followed using subtilisin from bacillus licheniformis as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-carboxylic acid-1,3-dioxolane methyl ester). The result is a β-anomer that has high diastereomeric purity.

Example 9: Enzymatic Resolution of α-anomer with Subtilisin from Bacillus Licheniformis.

The product acid is obtained from Example 8 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high steric purity.

Example 10: Enzymatic Resolution of β-anomer with Protease from Streptomyces Griseus.

The procedure of Examples 1-2 were followed using protease from streptomyces griseus as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-carboxylic acid-1,3-dioxolane methyl ester). The result is a β-anomer that has high diastereomeric purity.

Example 11: Enzymatic Resolution of α-anomer with Protease from Streptomyces Griseus.

The product acid is obtained from Example 10 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high steric purity.

Example 12: Enzymatic Resolution of β-anomer with Acylase from Aspergillus Melletus.

The procedure of Examples 1-2 were followed using acylase from aspergillus melletus as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-carboxylic acid-1,3-dioxolane methyl ester). The result is a β-anomer that has high diastereomeric purity.

Example 13: Enzymatic Resolution of α-anomer with Acylase from Aspergillus Melletus.

The product acid is obtained from Example 12 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high steric purity.

Example 14: Enzymatic Resolution of α-anomer with Proteinase from Bacillus Subtilis.

The procedure of Examples 1-2 were followed using proteinase from bacillus subtilis as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-carboxylicacid-1,3-dioxolane methyl ester). The result is a β-anomer that has high diastereomeric purity.

Example 15: Enzymatic Resolution of α-anomer with Proteinase from Bacillus Subtilis.

The product acid is obtained from Example 14 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high steric purity.

Example 16: Enzymatic Resolution of β-anomer with Diversa Clonezymes #5.

The procedure of Examples 1-2 were followed using diversa clonezymes #5 as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-carboxylic acid-1,3-dioxolanemethyl ester). The result is a β-anomer that has high diastereomeric purity.

Example 17: Enzymatic Resolution of α-anomer with Diversa Clonezymes #5.

The product acid is obtained from Example 16 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high steric purity.

Example 18: Enzymatic Resolution of β-anomer with Pronase from Streptomyces.

The procedure of Examples 1-2 were followed using pronase from streptomyces as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-carboxylic acid-1,3-dioxolane-methyl ester). The result is a β-anomer that has high diastereomeric purity.

Example 19: Enzymatic Resolution of α-anomer with Pronase from Streptomyces.

The product acid is obtained from Example 18 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high steric purity.

Example 20: Enzymatic Resolution of β-anomer with Lipase from Rhizopus Arrhizus.

The procedure of Examples 1-2 were followed using Lipase from rhizopus arrhizus as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-carboxylicacid-1,3-dioxolane methyl ester). The result is a β-anomer that has high diastereomeric purity.

Example 21: Enzymatic Resolution of α-anomer with Lipase from Rhizopus Arrhizus.

The product acid is obtained from Example 20 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high steric purity.

Example 22: Enzymatic Resolution of β-anomer with Lipoprotein Lipase from Pseudomonas Sp. Type B.

The procedure of Examples 1-2 were followed using lipoprotein lipase from pseudomonas sp. type B as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-carboxylic acid-1,3-dioxolane methyl ester). The result is a β-anomer that has high diastereomeric purity.

Example 23: Enzymatic Resolution of α-anomer with Lipoprotein lipase from Pseudomonas Sp. Type B.

The product acid is obtained from Example 22 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high steric purity.

Example 24: Enzymatic Resolution of β-anomer with Bacterial Proteinas .

The procedure of Examples 1-2 were followed using bacterial proteinase as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-carboxylicacid-1,3-dioxolane methyl ester). The result is a β-anomer that has high diastereomeric purity.

Example 25: Enzymatic Resolution of α-anomer with Bacterial Proteinase.

The product acid is obtained from Example 24 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high steric purity.

Example 26: Enzymatic Resolution of β-anomer with Lipase from Pseudomonas Cepacia.

The procedure of Examples 1-2 were followed using lipase from pseudomonas cepacia as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-carboxylic acid-1,3-dioxolane methyl ester). The result is a β-anomer that has high diastereomeric purity.

Example 27: Enzymatic Resolution of α-anomer with Lipase from Pseudomonas Cepacia.

The product acid is obtained from Example 26 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high steric purity.

Example 28: Enzymatic Resolution of β-anomer with Bacterial Proteinase.

The procedure of Examples 1-2 were followed using bacterial proteinase as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzoyloxymethyl)-4-carboxylicacid-1,3-diaxolane methyl ester). The result is a β-anomer that has high diastereomeric purity.

Example 29: Enzymatic Resolution of α-anomer with Bacterial Proteinase.

The product acid is obtained from Example 28 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high steric purity.

Example 30: Enzymatic Resolution of β-anomer with Cholesterol Esterase.

The procedure of Examples 1-2 were followed using cholesterol esterase as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzyloxymethyl)-4-carboxylicacid-1,3-diaxolane methyl ester). The result is a β-anomer that has high diastereomeric purity.

Example 31: Enzymatic Resolution of α-anomer with Cholesterol Esterase.

The product acid is obtained from Example 30 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high steric purity.

Example 32: Enzymatic Resolution of β-anomer with ESL-001-02.

The procedure of Examples 1-2 were followed using ESL-001-02 as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzyloxymethyl)-4-carboxylic acid-1,3-dioxolane methyl ester). The result is a β-anomer that has high diastereomeric purity.

Example 33: Enzymatic Resolution of α-anomer with ESL-001-02.

The product acid is obtained from Example 32 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high steric purity.

Example 34: Enzymatic Resolution of β-anomer with Horse Liver Esterase.

The procedure of Examples 1-2 were followed using horse liver esterase as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzyloxymethyl)-4-carboxylic acid-1,3-diaxolane methyl ester). The result is a β-anomer that has high diastereomeric purity.

Example 35: Enzymatic Resolution of α-anomer with Horse Liver Esterase.

The product acid is obtained from Example 34 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high steric purity.

Example 36: Enzymatic Resolution of β-anomer with Bovine Pancreatic Protease.

The procedure of Examples 1-2 were followed using bovine pancreatic protease as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzyloxymethyl)-4-carboxylic acid-1,3-dioxolane methyl ester). The result is a β-anomer that has high diastereomeric purity.

Example 37: Enzymatic Resolution of α-anomer with Bovine Pancreatic Protease.

The product acid is obtained from Example 36 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high steric purity.

Example 38: Enzymatic Resolution of β-anomer Protease from Streptomyces Caeopitosis.

The procedure of Examples 1-2 were followed using protease from streptomyces caeopitosis as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzyloxymethyl)-4-carboxylic acid-1,3-dioxolane methyl ester). The result is a β-anomer that has high diastereomeric purity.

Example 39: Enzymatic Resolution of α-anomer with Protease from Streptomyces Caeopitosis.

The product acid is obtained from Example 38 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high steric purity.

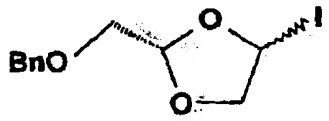
Example 40: Enzymatic Resolution of β-anomer with Substilisin from Bacillus Licheniformis.

The procedure of Examples 1-2 were followed using substilisin from bacillus licheniformis as the enzyme to separate a 2:1 (β:α) anomeric mixture of (2-(S)-benzyloxymethyl)-4-carboxylic acid-1,3-dioxolane methylester). The result is a β-anomer that has high diastereomeric purity.

Example 41: Enzymatic Resolution of α-anomer with Substilisin from Bacillus Licheniformis.

The product acid is obtained from Example 40 after it is dried with $MgSO_4$, filtered and concentrated *in-vacuo*. The α-anomer is isolated with high steric purity.

Example 42a: Preparation of 2S-Benzyloxymethyl-4R-iodo-1,3-dioxolane and 2S-Benzyloxymethyl-4S-iodo-1,3-dioxolane (Compound 42A).

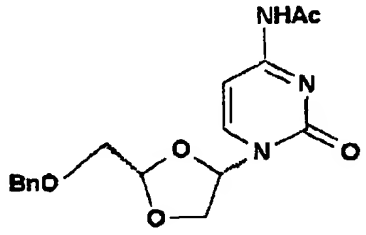


Compound 42A

A mixture consisting of 2S-benzyloxymethyl-4S acetoxy-1,3-dioxolane and 2S-benzyloxymethyl-4R-acetoxy-1,3-dioxolane in 1:2 ratio (6g; 23.8 mmol) was dried by azeotropic distillation with toluene *in-vacuo*. After removal of toluene, the residual oil was dissolved in dry dichloromethane (60 mL) and iodotrimethylsilane (3.55 mL; 1.05 eq.) was added at -78°C, under vigorous stirring. The dry-ice/acetone bath was removed after addition and the mixture was allowed to warm up to room temperature (15 min.). The product was 2S-benzyloxymethyl-4R-iodo-

1,3-dioxolane and 2S-benzyloxymethyl-4S-iodo-1,3-dioxolane.

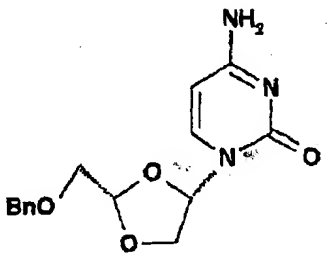
Example 43: Synthesis of β-L-4'-Benzyl-2'-deoxy-3'-oxa-N-4-acetyl-cytidine (compound 43A)



Compound 43A

The previously prepared iodo intermediate (Compound 42A) in dichloromethane, was cooled down to -78° C. Persylilated N-acetyl cytosine (1.1 eq) formed by reflux in 1,1,1,3,3,3-hexamethyl disilazane (HMDS) and ammonium sulphate followed by evaporation of HMDS was dissolved in 30 ml of dichloromethane and was added to the iodo intermediate. The reaction mixture was maintained at -78°C for 1.5 hours then poured onto aqueous sodium bicarbonate and extracted with dichloromethane (2x25mL). The organic phase was dried over sodium sulphate, the solid was removed by filtration and the solvent was evaporated *in-vacuo* to produce 8.1 g of a crude mixture. β-L-4'-benzyl-2'-deoxy-3'-oxacytidine and its α-L isomer were formed in a ratio of 5:1 respectively. This crude mixture was separated by chromatography on silica-gel (5% MEOH in EtOAc) to generate the pure β-L (cis) isomer (4.48 g). Alternatively, recrystallization of the mixture from ethanol produces 4.92 g of pure β isomer and 3.18 g of a mixture of β and α-isomers in a ratio of 1:1.

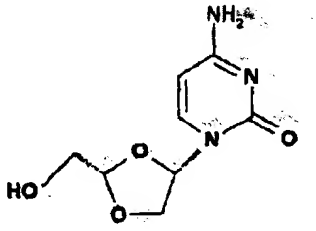
Example 44: Synthesis of β-L-4-Benzyloxy-2'-deoxy-3'-oxacytidine (Compound 44A)



Compound 44A

The protected β-L isomer (4.4 g) (Compound 43A) was suspended in saturated methanolic ammonia (250 mL) and stirred at room temperature for 18 hours in a closed vessel. The solvents were then removed *in-vacuo* to afford the deacetylated nucleoside in pure form.

Example 45: Synthesis of β-L-2'-deoxy-3'-oxacytidine (compound 45A)



Compound 45A

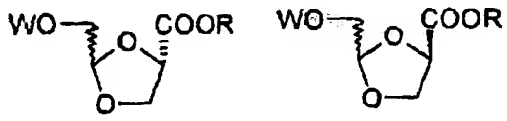
β-L-4'-Benzyl-2'-deoxy-3'-oxacytidine (Compound 44A) was dissolved in EtOH (200 mL) followed by addition of cyclohexene (6 mL) and palladium oxide (0.8 g). The reaction mixture was refluxed for 7 hours then it was cooled and filtered to remove solids. The solvents were removed from the filtrate by vacuum distillation. The crude product was purified by flash chromatography on silica-gel (5% MeOH in EtOAc) to yield a white solid

(2.33 g; 86% overall yield). α_D^{22} = -46.7° (c = 0.285; MeOH) m.p. = 192 - 194°C.

Some modifications and variations of the present invention including but not limited to selection of enzymes with high degree of sequence homology and optimization of reaction conditions will be obvious to a person of ordinary skill in the art from the foregoing detailed description of the invention. Such modifications and variations are intended to fall within the scope of one or more embodiments of the present invention as defined by the following claims.

What is claimed is:

1. A process for stereoselectively producing a dioxolane nucleoside analogue from an anomeric mixture of β and α anomers represented by the following formula A or formula B:



(A) (B)

wherein W is benzyl or benzoyl and R_1 is selected from the group consisting of C_{1-6} alkyl and C_{6-15} aryl, the process comprising:

hydrolyzing said mixture with an enzyme selected from the group consisting of cholesterol esterase, ESL-001-02, horse liver esterase, bovine pancreatic protease, α-chymotrypsin, protease from streptomyces caeopitosis, substilisin from bacillus licheniformis, protease from aspergillus oryzae, proteinase from bacilius licheniformis, subtilisin from bacillus licheniformis, protease from streptomyces griseus, acylase from aspergillus melletus, proteinase from bacillus subtilis, diversa clonezyme #5, pronase from streptomyces, lipase from rhizopus arrhizus, lipoprotein lipase from pseudomonas sp. type B, bacterial proteinase, lipase from pseudomonas cepacia and bacterial proteinase to stereoselectively hydrolyze predominantly one anomer to form a product wherein R_1 is replaced with H;

separating the product from unhydrolyzed starting material;

stereoselectively replacing the R group with a purinyl or pyrimidinyl or derivative thereof.

2. The process of claim 1, wherein the step of hydrolyzing results in the starting material having an anomeric purity of at least 80%.

3. The process of claim 1, wherein the step of hydrolyzing results in the starting material having an anomeric purity of at least 90%.

4. The process of claim 1, wherein the step of hydrolyzing results in the starting material having an anomeric purity of at least 95%.

5. The process of claim 1, wherein the step of hydrolyzing results in the starting material having an anomeric purity of at least 98%.

6. The process of claim 1, wherein the step of hydrolyzing results in the product having an anomeric purity of at least 80%.

7. The process of claim 1, wherein the step of hydrolyzing results in the product having an anomeric purity of at least 90%.

8. The process of claim 1, wherein the step of hydrolyzing results in the product having an anomeric purity of at least 95%.

9. The process of claim 1, wherein the step of hydrolyzing results in the product having an anomeric purity of at least 98%.

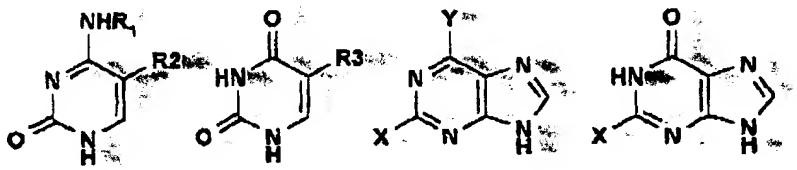
10. The process of claim 1, wherein W is benzyl and wherein the enzyme is selected from the group consisting of cholesterol esterase, ESL-001-02, horse liver esterase, bovine pancreatic protease, α-chymotrypsin, protease from streptomyces caeopitosis, substilisin from bacillus licheniformis.

11. The process of claim 10, wherein the enzyme is α-chymotrypsin.

12. The process of claim 10, wherein the enzyme is bovine pancreatic protease.

13. The process of claim 1, wherein W is benzoyl and wherein the enzyme is selected from the group consisting of protease from aspergillus oryzae, proteinase from bacillus licheniformis, subtilisin from Bacillus licheniformis, protease from streptomyces griseus, acylase from aspergillus melletus, proteinase from bacillus subtilis, diversa clonezymes #5, pronase from streptomyces, lipase from Rhizopus arrhizus, lipoprotein lipase from pseudomonas sp. type B, bacterial proteinase, lipase from pseudomonas cepacia.

14. The process of claim 1, wherein the purinyl or pyrimidinyl or derivative thereof is selected from the group consisting of:



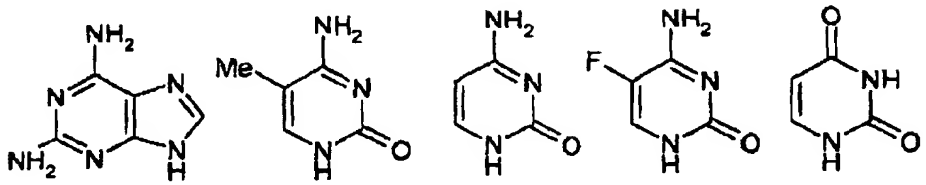
wherein

R_1 is selected from the group consisting of hydrogen, C_{1-6} alkyl, C_{1-6} acyl and RC(O) wherein R is hydrogen or C_{1-6} alkyl;

R_2 and R_3 are each independently selected from the group consisting of hydrogen, C_{1-6} alkyl, bromine, chlorine, fluorine, and iodine; and

X and Y are each independently selected from the group consisting of hydrogen, bromine, chlorine, fluorine, iodine, amino and hydroxyl.

15. The process of claim 1, wherein the purine or pyrimidine base or derivative thereof is selected from the group consisting of:



16. The process of claim 1, wherein the step of replacing further comprises:

acylating the second mixture to produce an acylated second mixture; and

glycosylating the acetylated second mixture with a purine or pyrimidine base or derivative thereof and a Lewis Acid to produce the dioxolane nucleoside analogue.